Human TLR1-9 Agonist Kit

Set of known agonists for human TLR1 to TLR9

Catalog code: tlrl-kit1hw

https://www.invivogen.com/human-tlr1-9-agonist-kit

For research use only

Version 23L05-MM

PRODUCT INFORMATION

Contents

- TLR1/TLR2 agonist - **Pam3CSK4** (10 µg)
- TLR2 agonist - **HKLM** (10^9 cells)
- TLR2/TLR6 agonist - **FSL-1** (10 µg)
- TLR3 agonist - **Poly(I:C) HMW** (500 µg)
- TLR3 agonist - **Poly(I:C) LMW** (500 µg)
- TLR4 agonist - **LPS-EK standard** (100 µg)
- TLR5 agonist - **FLA-ST standard** (10 µg)
- TLR7 agonist - **Imiquimod** (25 µg)
- TLR8 agonist - **ssRNA40/LyoVec™** (25 µg)
- TLR9 agonist - **ODN 2006** (100 µg - 12.98 nmol)
- 2 x 1.5 ml endotoxin-free water

Storage and stability

- Products are shipped at room temperature and should be stored according to the table below.

TLR Agonists	Store lyophilized product at	Store resuspended product for
Pam3CSK4	4°C	1 month at 4°C, 6 months at -20°C
HKLM	4°C	1 month at 4°C, 6 months at -20°C
FSL-1	4°C	6 months at 4°C
Poly(I:C) HMW	4°C	1 month at 4°C, 1 year at -20°C
Poly(I:C) LMW	4°C	1 month at 4°C, 1 year at -20°C
LPS-EK	-20°C	1 month at 4°C, 6 months at -20°C
FLA-ST	-20°C	6 months at -20°C
Imiquimod	-20°C	6 months at -20°C
ssRNA40/ LyoVec™	-20°C	1 week at 4°C
ODN 2006	-20°C	6 months at -20°C

Quality control

The biological activity of each agonist has been confirmed using cellular assays.

DESCRIPTION

• Pam3CSK4 - TLR1/TLR2 agonist

Pam3CSK4 is a synthetic tripalmitoylated lipopeptide that mimics the acylated amino terminus of bacterial lipoproteins. Pam3CysSerLys4 (Pam3CSK4) is a potent activator of the pro-inflammatory transcription factor NF-κB[1]. Recognition of Pam3CSK4 is mediated by TLR2 which cooperates with TLR1 through their cytoplasmic domain to induce the signaling cascade leading to the activation of NF-κB[2].
Molecular weight: 1852.33 g/mol

• HKLM - TLR2 agonist

HKLM is a freeze-dried heat-killed preparation of *Listeria monocytogenes* (LM), a facultative intracellular Gram-positive bacterium. Infection with LM induces a strong non-specific response characterized by the secretion of pro-inflammatory cytokines. This response is mediated by TLR2[3]. Stimulation with HKLM induces immediate activation of NF-κB and the production of pro-inflammatory cytokines[4].

• FSL-1 - TLR2/TLR6 agonist

FSL-1 (Pam2CGDPKHPKSF) is a synthetic lipoprotein that represents the N-terminal part of the 44-kDa lipoprotein LP44 of *Mycoplasma salivarium*[5]. The framework structure of FSL-1 is the same as that of MALP-2, a *Mycoplasma fermentans* derived lipopeptide (LP), but they differ in the amino acid sequence and length of the peptide portion[6]. FSL-1 is recognized by TLR2 and TLR6 inducing a MyD88-dependent signaling cascade that leads to the activation of NF-κB and the production of pro-inflammatory cytokines.
Molecular weight: 1666.2 g/mol

• Poly(I:C) HMW and Poly(I:C) LMW - TLR3 agonists

Poly(I:C) is a synthetic analog of double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Poly(I:C) is composed of a strand of poly(I) annealed to a strand of poly(C). The size of the strands varies. Poly(I:C) HMW has a high molecular weight (average size 1.5-8 kb), whereas Poly(I:C) LMW has a low molecular weight (average size 0.2-1 kb).
Poly(I:C) HMW and Poly(I:C) LMW may activate the immune system differently. dsRNA is known to induce interferons (IFNs) and other cytokines production. IFN induction is mediated by two different pathways. The first pathway leading to NF-κB activation depends on the dsRNA-responsive protein kinase (PKR)[7], whereas the second pathway is PKR-independent and involves TLR3[8].

• LPS from *E. coli* K12 (LPS-EK) - TLR4 agonist

Lipopolysaccharide (LPS), the major structural component of the outer wall of Gram-negative bacteria, is a potent activator of the immune system. LPS recognition is mediated by TLR4 which forms a complex with MD2 and CD14[9] leading the production of pro-inflammatory cytokines through the MyD88 pathway. LPS signaling also involves a MyD88-independent cascade that mediates the expression of IFN-inducible genes via the adaptor protein TRIF[10].

• Flagellin from *S. typhimurium* (FLA-ST) - TLR5 agonist

Flagellin is the major component of the bacterial flagellar filament, which confers motility on a wide range of bacterial species. Flagellin is a potent stimulator of innate immune responses in a number of eukaryotic cells and organisms, including both mammals and plants. In mammals, flagellin is recognized by TLR5[11] and triggers defense responses both systemically and at epithelial surfaces. Flagellin induces the activation of NF-κB and the production of cytokines and nitric oxide depending on the nature of the TLR5 signaling complex[12].

TECHNICAL SUPPORT
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• Imiquimod - TLR7 agonist

Imiquimod (R837), an imidazoquinoline amine analogue of guanosine, is an immune response modifier with potent indirect antiviral activity. Imiquimod is an approved treatment for external genital warts caused by human papillomavirus infection. This low molecular synthetic molecule induces the production of cytokines such as IFN-α. Unlike R848, Imiquimod activates only TLR7 but not TLR8[13]. This activation is MyD88-dependent and leads to the induction of the transcription factor NF-κB[14].

Molecular weight: 276.8 g/mol

• ssRNA40/LyoVec™ - TLR8 agonist

ssRNA40/LyoVec™ is a 20-mer phosphothioate protected single-stranded RNA (ssRNA) oligonucleotide containing a GU-rich sequence[15]. ssRNA40 is complexed with the cationic lipid LyoVec™ (ratio 1:2), to protect it from degradation and facilitate its uptake, and lyophilized to generate ssRNA40/LyoVec™. When complexed to cationic lipids, ssRNA can substitute for viral RNAs in inducing TNF-α and IFN-α production in peripheral blood mononuclear cells[15, 16]. ssRNA40 complexes are recognized by TLR8 in humans and TLR7 in mice.

5'-GCCCGUCUGUUGUGUGACUC-3' (phosphorothioate bases)

• ODN 2006 (ODN 7909) - TLR9 agonist

CpG ODNs are synthetic oligonucleotides containing unmethylated CpG dinucleotides in particular sequence contexts that induce strong immunostimulatory effects through the activation of TLR9[17, 18]. ODN 2006 (also known as ODN 7909 or PF-3512676) is a CpG ODN class B with a preference for human TLR9. B-class CpG ODNs contain a full phosphorothioate backbone with one or more CpG dinucleotides. They strongly activate B cells but weakly stimulate IFN-α secretion.

5'- tcg tcg ttt tgt cgt ttt gtc gtt -3' (phosphorothioate bases)

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METHODS

Preparation of TLR agonist stock solutions

Product	Working concentration	Stock solution concentration	Volume of solvent
Pam3CSK4	0.1-10 ng/ml	100 µg/ml	100 µl H_2O
HKLM	10^7-10^8 cells/ml	10^{10} cells/ml	100 µl H_2O
FSL-1	0.01-100 ng	100 µg/ml	100 µl H_2O
Poly(I:C) HMW	30 ng-10 µg/ml	1 mg/ml	500 µl H_2O*
Poly(I:C) LMW	30 ng-10 µg/ml	1 mg/ml	500 µl H_2O
LPS-EK	1 ng-10 µg/ml	100 µg/ml	1 ml H_2O
FLA-ST	10 ng-10 µg/ml	100 µg/ml	100 µl H_2O
Imiquimod	1-5 µg/ml	100 µg/ml	250 µl H_2O
ssRNA40/ LyoVec™	0.25-5 µg/ml	100 µg/ml	250 µl H_2O
ODN 2006	1-5 µM	500 µM	26 µl H_2O

**Note: Following resuspension of Poly(I:C) HMW, heat the solution for 10 minutes at 65-70°C, then allow the solution to cool at room temperature for 1 hour to ensure proper annealing.*

TLR activation

Activation of TLRs can be monitored using InvivoGen's HEK-Blue™ TLR reporter cells. These cells stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) and overexpress a TLR gene. For more information visit: https://www.invivogen.com/hek-blue-tlr-cells.

1. Prepare HEK-Blue™ TLR cell suspension according to the data sheet.
2. Incubate cells with the corresponding agonist for 6-24 h at 37 °C, 5% CO_2.
3. Determine TLR stimulation by assessing cytokine expression using an ELISA, or SEAP expression using a SEAP detection medium, such as HEK-Blue™ Detection.

RELATED PRODUCTS

Product	Description	Cat. Code
FLA-ST	TLR5 agonist	tlrl-stfla
FSL-1	TLR2/TLR6 agonist	tlrl-fsl
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ hTLR2 cells	TLR2 reporter cells	hkb-htlr2
HEK-Blue™ hTLR3 cells	TLR3 reporter cells	hkb-htlr3
HEK-Blue™ hTLR4 cells	TLR4 reporter cells	hkb-htlr4
HEK-Blue™ hTLR5 cells	TLR5 reporter cells	hkb-htlr5
HEK-Blue™ hTLR7 cells	TLR7 reporter cells	hkb-htlr7
HEK-Blue™ hTLR8 cells	TLR8 reporter cells	hkb-htlr8
HEK-Blue™ hTLR9 cells	TLR9 reporter cells	hkb-htlr9
HKLM	TLR2 agonist	tlrl-hklm
Imiquimod (R837)	TLR7 agonist	tlrl-imqs
LPS-EK	TLR4 agonist	tlrl-eklps
ODN 2006 (ODN 7909)	TLR9 agonist	tlrl-2006
Pam3CSK4	TLR1/TLR2 agonist	tlrl-pms
Poly(I:C) HMW	TLR3 agonist	tlrl-pic
Poly(I:C) LMW	TLR3 agonist	tlrl-picw
ssRNA40/LyoVec™	TLR8 agonist	tlrl-lrna40

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